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HER2 Overexpression and Paclitaxel Sensitivity in Breast Cancer: Therapeutic Implications

ABSTRACT

Overexpression by the HER2 gene plays a significant role in breast cancer pathogenesis, and the phenomenon is commonly regarded as a predictor of a poor prognosis. HER2 overexpression has been linked to sensitivity and/or resistance to hormone therapy and chemotherapeutic regimens, including CMF (cyclophosphamide, methotrexate, and fluorouracil) and anthracyclines. Studies of patients with advanced disease demonstrate that, despite the association of HER2 overexpression with poor prognosis, the odds of HER2-positive patients responding clinically to taxanes were greater than three times those of HER2-negative patients. Further studies in preclinical models used combination therapy for breast cancer cells that overexpress HER2, and the use of agents that interfere with HER2 function plus paclitaxel (Taxol) resulted in significant antitumor effects.

The taxanes are an important new class of anticancer agents with a unique mechanism of action.[1] Paclitaxel (Taxol), the first taxane used in clinical trials, was originally isolated in 1971 from the bark of the Pacific yew, *Taxus brevifolia*. It was selected for clinical development based on impressive antitumor activity against the implanted B16 melanoma and the human MX-1 mammary tumor xenograft.

Since then, paclitaxel has been shown to have a high degree of antitumor activity in women with metastatic breast cancer, as well as a lack of cross-resistance with anthracyclines. Current clinical research with paclitaxel in breast cancer is focused on several aspects, including: optimal dosing and scheduling; the agent's role in the treatment of early breast cancer; its use in high-dose intensity therapy of breast cancer; and combination therapy of paclitaxel with other antineoplastic agents.

An area of increasing interest in clinical research on taxanes is the possible role of oncogenes, such as HER2, in determining clinical response to paclitaxel. Studies have examined whether strategies can be designed to increase the agent's efficacy (or curb resistance to it) in breast cancers that overexpress HER2. Available data that will be presented in this review suggest that HER2 overexpression may influence the response to paclitaxel in patients with metastatic breast cancer and that anti-HER2 monoclonal antibodies significantly increase the antitumor activity of paclitaxel in vitro and in vivo.

HER2/c-erbB-2/neu in Breast Cancer

During the last decade, proto-oncogenes encoding growth factors and growth factor receptors have been found to play important roles in the pathogenesis of several human malignancies, including breast cancer.[2] The HER2 gene (also known as *neu* and as *c-erbB-2*) encodes a 185-kD transmembrane glycoprotein receptor ($p185^{HER2}$) that has partial homology with the epidermal growth factor (EGF) receptor: the gene shares intrinsic tyrosine kinase activity with the receptor.[3-5] The EGF receptor and $p185^{HER2}$ belong to a superfamily of receptors known as type I tyrosine kinase receptors, which have an extracellular ligand-binding domain, a transmembrane lipophilic segment, and an intracellular protein tyrosine kinase domain with a regulatory carboxyl terminal segment.[6]

HER2 is overexpressed in 25% to 30% of human breast cancers[7,8] and indicates a worse prognosis in patients who have positive axillary lymph nodes.[6,7,9] The observation that HER2 overexpression is associated with a poor prognosis could imply that HER2 is solely a marker event. On the other hand, HER2 could be a prognostic factor that plays a role in the pathogenesis of breast cancer

Role of Monoclonal Antibodies

Several lines of evidence support a direct role for HER2 in the pathogenesis and clinical aggressiveness of overexpressing tumors: (1) The introduction of HER2 into nonneoplastic cells causes their malignant transformation.[10,11] (2) Transgenic mice expressing HER2 develop mammary tumors.[12] (3) HER2 overexpression is common in ductal carcinomas in situ and in their associated invasive cancers.[13,14] (4) The mechanisms responsible for this growth advantage are thought to be related to the fact that $p185^{HER2}$ overexpression results in activation of a series of signaling pathways (PLC-gamma/phosphatidylinositol; PI(3)kinase; STAT91/ISGF-3; ras/raf/MAP-kinase pathway; src family), and that activation of these pathways results in gene activation that ultimately results in cell proliferation.[6] (5) Antibodies directed against $p185^{HER2}$ can inhibit the growth of tumors and of transformed cells that express high levels of this receptor.[15-19]

The latter observation suggests that $p185^{HER2}$ may be a potential target for the treatment of breast cancer or preinvasive breast lesions because these cells commonly overexpress HER2. The murine monoclonal antibody (MoAb) 4D5, directed against the extracellular domain of $p185^{HER2}$ (ECD^{HER2}), is a potent inhibitor of in vitro growth and, in xenograft models, of human breast cancer cells overexpressing HER2.[20-22] Murine MoAbs, however, are limited clinically because they are immunogenic. Therefore, to facilitate further clinical investigations, MoAb 4D5 was humanized. The resulting recombinant humanized anti-$p185^{HER2}$ MoAb (rhuMoAb HER2) was found to be safe and to have dose-dependent pharmacokinetics in two prior phase I clinical trials.

HER2 Overexpression as a Predictor of Response to Taxanes

Since HER2 plays a role in breast cancer pathogenesis and HER2 overexpression correlates with more aggressive clinical behavior, several studies have attempted to correlate whether HER2 overexpression is a predictor for response to systemic therapy. Studies to date have shown that HER2 overexpression predicts a worse response to hormonal therapy with tamoxifen (Nolvadex) in advanced disease[23,24] and in early-stage breast cancer patients.[25]

Dose-Response Effects to Anthracyclines

The relationship between HER2 overexpression and response to chemotherapy appears to be more complex. Data indicate that HER2-positive tumors have increased resistance to adjuvant CMF (cyclophosphamide, methotrexate, and fluorouracil)-based therapy[26,27] and, conversely, increased dose-response effects to an anthracycline-containing regimen.[28]

In Intergroup Study 0011,[26] patients with primary breast cancer tumors larger than 3 cm or with estrogen receptor-negative tumors were randomized to receive either CMF chemotherapy or observation. Patients with HER2-negative tumors who received adjuvant chemotherapy showed significantly improved disease-free survival when compared with untreated patients. In contrast, patients with HER2-positive tumors showed no benefit from adjuvant therapy.

In the International Breast Cancer Study Group trial, patients with node-positive early breast cancer were randomized to receive either one cycle of perioperative chemotherapy or prolonged adjuvant chemotherapy, defined as six cycles of CMF-based chemotherapy.[27] In this study, the effect of prolonged chemotherapy was greater in patients with HER2-negative tumors.

The possible predictive role of HER2 overexpression has also been analyzed in patients with early breast cancer treated with anthracycline-containing adjuvant therapy. In a well-known randomized study by the Cancer and Leukemia Group B (CALGB), three doses (high, moderate, and low) of cyclophosphamide, doxorubicin, and fluorouracil were compared in women with node-positive breast cancer.[28] Patients randomly assigned to the high-dose regimen of adjuvant chemotherapy had significantly longer disease-free and overall survival if their tumors overexpressed HER2. This dose-response effect was not observed in patients whose tumors had minimal or no HER2 expression.

Thus, findings from the CALGB study suggest that there is a significant dose-response effect of adjuvant therapy with an anthracycline-containing regimen in patients with HER2 overexpression but not in patients with no or minimal HER2 expression. The group's final conclusion was that HER2 over-

expression may be a useful marker for identifying patients who are most likely to benefit from high doses of adjuvant doxorubicin-based chemotherapy.

HER2 Overexpression and Taxane Sensitivity

Because taxanes are becoming widely used in the management of advanced breast cancer, we decided to analyze whether there is a relationship between HER2 expression and clinical sensitivity to taxanes. In a study performed at Memorial Sloan-Kettering Cancer Center, the possible relationship between HER2 overexpression and response to taxanes was analyzed in patients with metastatic breast cancer.[29] HER2 expression was studied in patients treated with one of eight protocols of single-agent taxane therapy over the past 5 years. All patients had bidimensionally measurable disease and histologically confirmed metastatic breast cancer.

Archived paraffin-embedded tumor tissue was available for immunohistochemistry in 122 patients out of the total of 212 patients treated: of these, 102 (84%) received paclitaxel and 20 (16%), docetaxel (Taxotere). Geographic considerations were the most frequent obstacle in lack of tissue availability for HER2 analysis.

Tumor expression of HER2 was determined by immunohistochemical analysis of a set of thin sections prepared from patients' paraffin-archived tumor blocks (as previously described[7,8]). The primary detecting antibody used was murine MoAb 4D5, directed at the extracellular domain of $p185^{HER2}$. Tumors were considered to overexpress HER2 if at least 10% of the tumor cells exhibited characteristic membrane staining for $p185^{HER2}$.

Seven prognostic factors were assessed for association with tumor response: HER2 overexpression, scored as positive or negative; estrogen-receptor status; extent of disease, divided into one to two involved organ systems vs three or more involved systems; extent of prior chemotherapy, categorized as one to two courses or more than two courses; presence of visceral disease; prior therapy with doxorubicin; and Karnofsky performance status, stratified as 60 to 80 or 90 to 100.

In 37.7% of patients, tumors were positive by immunohistochemistry with the 4D5 antibody. The overall response to taxanes for all patients in this analy-

sis was 46.7%. Remarkably, 65.2% of patients with HER2-positive tumors responded vs 35.5% of patients with HER2-negative tumors. Using a Mantel-Haenszel test, the P value for this difference was significant at .002. Visceral dominance (P = .011), low performance status (P = .057), and extensive prior therapy correlated with poor clinical response. Among these, HER2 overexpression was positively correlated with low performance status (P = .002), and low performance status with extensive prior therapy.

These correlations should bias against response in HER2-positive cases, which was not observed. Indeed, stratified analysis controlling for confounding variables demonstrated the value of HER2 status in predicting taxane response. The odds ratios of response for HER2-positive vs HER2-negative tumors were 3.95 after adjusting for visceral disease, 3.17 after adjusting for number of prior therapies, and 3.06 after adjusting for performance status. Thus, despite a positive correlation of HER2 expression and poor prognostic features, the odds of HER2-positive patients responding clinically to taxanes were greater than three times those of HER2-negative patients.

Tentative Results With Polyclonal Antibody

Tumor specimens were also analyzed with a rabbit polyclonal antibody directed at the cytoplasmic c-terminus epitope of p185^{HER}. Immunohistochemical evaluation with this antibody resulted in a higher proportion of HER2 positivity (57%). Patients who were shown to have HER2-positive tumors using this antibody were more likely to respond to taxanes, although the difference was not statistically significant (P = .3).

Results with this polyclonal antibody have to be viewed with caution because 57% of the tumors stained positive for HER2 overexpression—a higher proportion than with the MoAb, and higher than has been reported previously. Further confirmatory studies are needed to verify our results, which could have significant implications for the treatment of patients with advanced breast cancer.

Paclitaxel and the Signal Transduction Pathway

The possible mechanisms underlying the interaction between HER2 overexpression and taxane sensitivity are
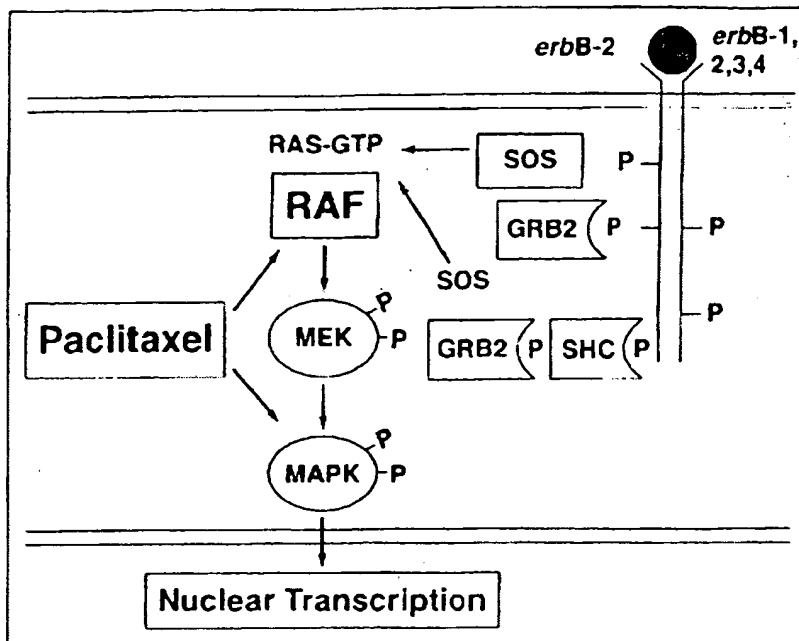

Figure 1: Interaction of Paclitaxel with p185^{HER} Signal Transduction Pathway—Paclitaxel activates c-raf-1 and MAP kinase in breast cancer cells.

unknown Paclitaxel stabilizes microtubules, prevents tubulin depolymerization, and promotes tubulin bundling. In addition to this well-documented mechanism of action, some evidence suggests that paclitaxel activates key elements of the HER2 signal transduction pathway (Figure 1).[30-32]

The mitogen-stimulated protein serine/threonine kinase c-raf-1 functions as a central component of the mitogen-activated protein kinase (MAP kinase) signal transduction pathway.[33] In MCF-7 breast cancer cells, paclitaxel treatment leads to activation of c-raf-1, documented by a reduced c-raf-1 electrophoretic mobility after paclitaxel exposure.[30] Furthermore, paclitaxel therapy induces a dose- and time-dependent accumulation of the cyclin inhibitor of p21^{WAF1}, and c-raf-1 depletion prevents this activation. In addition to c-raf-1 activation, tyrosine phosphorylation of MAP kinase is a well-documented response to paclitaxel, and it probably represents a functionally important event by the agent.[30,32]

The activation of the HER2 signal transduction pathway by paclitaxel could also result in activation of paclitaxel-induced apoptosis.[31] Thus, HER2 overexpression would provide

an increased opportunity to enhance the cytotoxic effects of paclitaxel.

Combined Therapy With Anti-HER2 Agents and Chemotherapy

As already mentioned, p185^{HER} represents a potential target for tumors that overexpress HER2. A novel approach that is currently being explored is the combined use of therapies directed at p185^{HER}, such as MoAbs given alone or in combination with conventional chemotherapeutic agents, including paclitaxel.

Several groups have produced antibodies directed against the p185^{HER} receptor protein on human cells. Some of these antibodies can inhibit the growth of monolayer cultures of breast and ovarian tumor cells that overexpress p185^{HER}.[15-18,20,34,35] In extensive studies conducted at Genentech, Inc, a clear relationship between the level of HER2 proto-oncogene expression and sensitivity to the growth-inhibitory effects of the antibodies was observed.[20,21,34]

Breast carcinoma cells with little ex-

pression of p185[HER2] (eg. MCF7, MDA-MB-231, ZR-75-1, and MDA-MB-436) were not inhibited by MoAbs. Cell lines with higher levels of p185[HER2] (MDA-MB-175, MDA-MB-453, MDA-MB-361) were increasingly more sensitive to antibody-mediated growth inhibition. SK-BR-3 and BT-474, the highest expressors of p185[HER2] of the cell lines studied, were the most sensitive to the antiproliferative effects (showing approximately 70% growth inhibition).

The most potent growth inhibitory anti-p185[HER2] MoAb was 4D5. The activity of MoAb 4D5 and a humanized version of this MoAb against human breast adenocarcinoma cells bearing HER2/c-erbB-2 has been evaluated in the nude mouse xenograft model. Inhibition of tumor growth has been observed[36,37] with eradication of well-established tumors.[37] Thus, MoAb 4D5 appears to have potential therapeutic applications for tumors overexpressing p185[HER2].

A "Humanized" Antibody

In an attempt to circumvent an antiglobulin response during therapy, a "humanized" antibody was constructed by Genentech scientists.[38] Denoted rhuMoAb HER2, this antibody contained the antigen-binding portions of murine MoAb 4D5 (discussed above) and a human immunoglobulin variable region framework. rhuMoAb HER2 has potency comparable to murine 4D5 in blocking the proliferation of breast carcinoma cells in vitro. Furthermore, rhuMoAb HER2 IgG1 is much more efficient in supporting antibody-dependent cellular cytotoxicity, which could increase its antitumor activity.

Initial phase I studies were conducted, and a phase II study has recently been completed in patients with metastatic breast carcinomas overexpressing HER2 [39] Forty-six patients were treated with a loading dose of 250 mg of IV rhuMoAb HER2, then with 10 weekly doses of 100 mg each. Patients with no progression of disease at the completion of this treatment period were offered a weekly maintenance phase of 100 mg.

The study patients had extensive metastatic disease, and most had re-

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ceived extensive prior anticancer therapy. Adequate serum levels of rhuMoAb HER2 were obtained in 90% of the patients. The mean serum half-life of rhuMoAb HER2 was 8.3 ± 5.0 days.

Interestingly, rhuMoAb HER2 serum half-life was found to be dependent on the presence of circulating p185[HER2] receptor extracellular domain ECD[HER2] released from the tumor into the serum. In those patients with circulating levels of tumor-shed ECD[HER2] in serum that were > 500 ng/mL, rhuMoAb HER serum half-lives were shorter, and therapeutic trough levels of rhuMoAb HER2 were not achieved. A likely explanation for these observations is that, in the presence of ECD[HER2] in the serum, antigen-antibody complexes form and are rapidly cleared from the circulation.

Toxicity was minimal, and no antibodies against rhuMoAb HER2 were detected in any patient. Objective responses were seen in 5 of the 43 evaluable patients: 1 complete remission and 4 partial remissions (overall response rate, 11.6%; 95% CI, 4.36% to 25.9%). Responses were observed in liver, mediastinum, lymph nodes, and chest wall lesions. Minor responses, seen in 2 patients, and stable disease, occurring in 14 patients, lasted for a median of 5.1 months. One patient is still in pathologically confirmed complete remission 72 months after starting therapy.

Thus, rhuMoAb HER2 is clinically active in patients who have metastatic breast cancers that overexpress HER2 and have received extensive prior therapy. A confirmatory study that will include 200 patients with less heavily pretreated metastatic disease is currently underway.

Anti-HER MoAb Combined With Chemotherapy

In order to enhance the antitumor activity of anti-p185[HER2] MoAb, several investigators have used these antibodies in combination with chemotherapeutic agents. Hancock et al[40] used the MoAb TAB 250, specific to an extracellular epitope of the p185[HER2] protein, in combination with cisplatin (Platinol) against human breast carcinoma SKBR-3 cells and ovarian carcinoma SKOV-3 cells. They showed that MoAb TAB 250 markedly enhanced the antitumor effects of cisplatin both in vitro and in vivo.

Using the same antibody, Arteaga has shown enhanced etoposide (VePesid)-induced cytotoxicity against human

breast carcinoma cells and postulated that p185[HER2] antibodies may alter the sensitivity of topoisomerase II toward etoposide.[41] Slamon, in studies using MoAb 4D5, has demonstrated that 4D5 enhances sensitivity to cisplatin in cisplatin-resistant ovarian carcinoma cell lines. A possible mechanism is 4D5's interference with the repair of cisplatin-induced DNA damage, thus promoting cisplatin cytotoxicity.[36]

Paclitaxel/Doxorubicin/rhuMoab HER2 Regimen

We have explored the activity of paclitaxel and doxorubicin, the two chemotherapeutic agents most active against breast cancer, in combination with rhuMoAb HER2. Studies of the combined therapy with 4D5 were conducted in monolayer cell culture soft agar, as well as with breast cancer human tumor xenografts in nude mice. For the in vivo studies, 10^7 BT-474 cells, which express high levels of p185[HER2], were grown subcutaneously to a mean size of 200 mm³ over 11 days. Animals were then treated with MoAb 4D5 alone at a dose of .3 mg/kg intraperitoneally (IP) twice a week, paclitaxel alone at a dose of 20 mg/kg IV, or both therapies combined.

Therapy with MoAb 4D5 alone produced a 35% growth inhibition, and paclitaxel alone resulted in a 35% growth inhibition when compared with animals treated with a control MoAb. The treatment with paclitaxel plus 4D5 resulted in major antitumor activity, with 93% inhibition of growth. This result was markedly better than an equipotent dose of doxorubicin (10 mg/kg IP) and 4D5 (70% inhibition). In addition, paclitaxel combined with 4D5 resulted in the disappearance of well-established xenografts.[37]

Cisplatin/rhuMoAb HER2 Therapy

In parallel with the phase II clinical trial described above, which used rhuMoAb HER2 alone, a phase II study of rhuMoAb HER2 in combination with cisplatin has been conducted in patients with breast carcinomas that overexpress p185[HER2] and a history of proven refractoriness to chemotherapy.[42] Thirty-six patients were treated with an IV loading dose of 250 mg of rhuMoAb HER2 on day 0, followed by weekly administration of 100 mg of MoAb in combination with cisplatin, 75 mg/m², on day 1 and every 3 weeks thereafter

In this group of patients with expected cisplatin resistance, the observed response rate to the combined therapy was 25%, suggesting that the synergy observed in the laboratory was reproducible in the clinic. In addition, the combined therapy was no more toxic than cisplatin alone.

Phase III Study of rhuMoAB HER2 Combined With Chemo

Results from the phase II studies and the activity of rhuMoAb HER2 against xenografts when given in combination with doxorubicin and paclitaxel have been encouraging. These positive results have led to the design of a phase III multinational study of chemotherapy in combination with rhuMoAb HER2 in patients with HER2-overexpressing breast tumors who have not received prior chemotherapy for metastatic disease (Figure 2). In addition to proven HER2 overexpression by immunohistochemistry, eligible patients must have measurable disease and must not have received prior chemotherapy for metastatic disease.

In this ongoing study, patients are randomized to one of two treatment arms: the active arm, which consists of rhuMoAb HER2 in combination with cytotoxic chemotherapy; or the control arm, which consists of cytotoxic chemotherapy alone. Patients who are randomized to the rhuMoAb HER2 arm receive weekly administration of the antibody at a dose similar to the phase II studies. After the completion of cytotoxic chemotherapy, patients assigned to the HER2 arm continue with weekly rhuMoAb HER2 administration until disease progression. Patients in the control arm are given the option of receiving rhuMoAb HER2 when disease progression occurs.

Patients receive one of two chemotherapy regimens for a minimum of six cycles: cyclophosphamide and doxorubicin or epirubicin, if patients have not received anthracycline therapy in the adjuvant setting; or paclitaxel, if patients have received anthracycline therapy in the adjuvant setting. Because anthracyclines are widely used in the adjuvant setting, it is likely that a significant number of patients will be treated with paclitaxel ± rhuMoAb HER2. The main goal of this study is to determine whether the addition of this anti-HER2 antibody increases the time to disease progression compared with the group of patients treated with antibody alone.
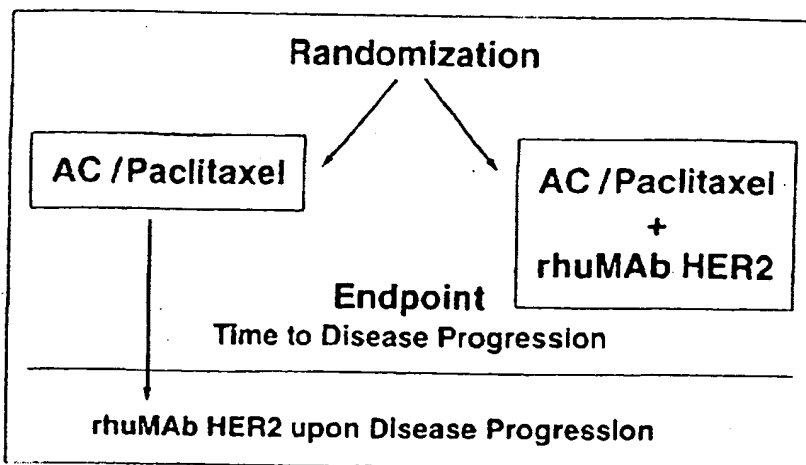

Randomization

AC / Paclitaxel

AC / Paclitaxel
+
rhuMAb HER2

Endpoint
Time to Disease Progression

rhuMAb HER2 upon Disease Progression

Figure 2: Treatment Schema of the Phase III Randomized Multicenter Study— Treatment consists of chemotherapy ± rhuMoAb HER2 in patients with metastatic breast tumors that overexpress HER2. Patients are randomized to the active arm, which consists of rhuMoAb HER2 in combination with cytotoxic chemotherapy (either Adriamycin and cyclophosphamide [AC] or paclitaxel), or the control arm, which consists of cytotoxic chemotherapy alone (either AC or paclitaxel). rhuMoAb HER2 is given weekly until disease progression occurs. Patients in the control arm are given the option of receiving rhuMoAb HER2 when disease progression occurs. The study end point is time to disease progression.

Future Directions

An alternative approach to interfering with the function of type I tyrosine kinase receptors, including p185^{HER2}, is direct inhibition of the receptor protein tyrosine kinase. These enzymes catalyze the transfer of the terminal phosphate from adenosine triphosphate (ATP) to the phenolic group of tyrosine in substrate proteins. Erbstatin, synthetic compounds known as tyrphostins, and the 2-thio-indoles inhibit the tyrosine kinase activity of the type I receptors by competing with ATP.[43-45] Once the activity of these agents has been defined, a logical next step would be to combine them with paclitaxel in vitro and in vivo.

To explore the potential anticancer effects of these compounds, we have studied the 2-thio-indole, PD153035, a reversible inhibitor of the EGF receptor tyrosine kinase and, to a lesser degree, of the p185^{HER2} receptor.[46] PD15035 completely inhibited phosphorylation of the EGF receptor and p185^{HER2} tyrosine kinase and prevented anchorage-dependent and -independent growth. In addition, a close correlation was observed between higher receptor number and greater growth inhibition.

It is likely that these agents may be

able to increase the antitumor activity of paclitaxel in a similar fashion to that observed with anti-HER2 antibodies. Support for this concept is provided by M-C Hung and colleagues in their studies with emodin, a protein tyrosine kinase inhibitor that inhibits p185^{HER2} tyrosine kinase activity.[47] In non-small-cell lung cancer cells that overexpress HER2, they observed a marked synergism of emodin given in combination with chemotherapy.

Overexpression of p185^{HER2} results in activation of a series of signaling pathways, including the ras/raf/MAP-kinase pathway. Peptidomimetic inhibitors of farnesyl:protein transferase that selectively block farnesylation of cellular proteins, including p21ras, inhibit ligand-induced stimulation of MAP-kinase in breast cancer cells and also have the potential for combination with paclitaxel in preclinical models.

Conclusions

The predictive value of HER2 overexpression for paclitaxel response requires independent confirmation in advanced disease and early breast cancer. This question could be addressed in ongoing studies of paclitaxel-based adjuvant therapy

in breast cancer. In preclinical models, the combined therapy of breast cancer cells that overexpress HER2 with agents that interfere with HER2 function and paclitaxel results in a marked antitumor effect. One such strategy, the combination of anti-HER2 MoAb with paclitaxel, is currently being evaluated. If the results of these studies are positive, we might be faced with a novel paradox, in which expression of a receptor that confers a worse prognosis provides us with an opportunity for increased response to taxanes.

References

1. Rowinsky EK, Donehower RC: Drug therapy: paclitaxel (Taxol). *N Engl J Med* 332:1004-1014, 1995.

2. Aaronson SA: Growth factors and cancer. *Science* 254:1146-1153, 1991.

3. Coussens L, Yang-Feng TL, Liao Y-C, et al: Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 230:1132-1139, 1985.

4. Akiyama T, Sudo C, Ogawara H, et al: The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 232 1644-1646, 1986.

5. Stern DF, Hefferman PA, Weinberg RA: p185, a product of the neu proto-oncogene, is a receptorlike protein associated with tyrosine kinase activity. *Mol Cell Biol* 6:1729-1740, 1986.

6. Hynes NE, Stern DF: The biology of erB-2/neu/HER-2 and its role in cancer. *Biochemica et Biophysica Acta* 1198:165-184, 1994.

7. Slamon DJ, Godolphin W, Jones LA, et al: Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 244:707, 1989.

8. Slamon DJ, Clark GM, Wong SG, et al: Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene. *Science* 235:177-182, 1987.

9. Ravdin PV, Chamness GC: The c-erbB-2 proto-oncogene as a prognostic and predictive marker in breast cancer: a paradigm for the development of other macromolecular markers—a review. *Gene* 159:19-27, 1995.

10. Hudziak RM, Schlessinger J, Ullrich A: Increased expression of the putative growth factor receptor p185HER1 causes transformation and tumorigenesis of NIH 3T3 cells. *Proc Natl Acad Sci USA* 84:7159-7163, 1987.

11. Di Fiore PP, Pierce JH, Kraus MH, et al: erbB-2 is a potent oncogene when overexpressed in NIH 3T3 cells. *Science* 1987; 237:178-182.

12. Guy CT, Webster MA, Schaller M, et al: Expression of the neu protooncogene in the mammary epithelium of transgenic mice induces metastatic disease. *Proc Natl Acad Sci USA* 89:10578-10582, 1992.

13. Gusterson BA, Machin LG, Gullick WJ, et al: Immunohistochemical distribution of c-erbB-2 in infiltrating and in situ breast cancer. *Int J Cancer* 42:842-845, 1988.

14. van de Vijver MJ, Peterse JL, Mooi WJ, et al: NEU-protein overexpression in breast cancer—association with comedo-type ductal carcinoma in situ and limited prognostic value in stage II breast cancer. *N Engl J Med* 319:1239-1245, 1988.

15. Drebin JA, Link VC, Stern DF, et al: Down-modulation of an oncogene protein product and reversion of the transformed phenotype by mono-clonal antibodies. *Cell* 41:695-706, 1985.

16. McKenzie SJ, Marks PJ, Lam T, et al: Generation and characterization of monoclonal antibodies specific for the human neu oncogene product, p185. *Oncogene* 4:543-548, 1989.

17. Stancovski I, Hurwitz E, Leitner D, et al: Mechanistic aspects of the opposing effects of monoclonal antibodies to the erbB-2 receptor on tumor growth. *Proc Natl Acad Sci USA* 88:8691, 1991.

18. Hancock MC, Langton BC, Chan T, et al: A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 51:4575-4580, 1991.

19. Harwerth IM, Wels W, Schlegel J, et al: Monoclonal antibodies directed to the erbB-2 receptor inhibit in vivo tumour cell growth. *Br J Cancer* 68:1140-1145, 1993.

20. Hudziak RM, Lewis GD, Winget M, et al: p185HER2 Monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol* 9:1165-1172, 1989.

21. Lewis GD, Figari I, Fendly B, et al: Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. *Cancer Immunol Immunother* 37:255-263, 1993.

22. Shepard HM, Lewis GD, Sarup JC, et al: Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic (review). *J Clin Immunol* 11:117-127, 1991.

23. Klijn JGM, Berns EMJJ, Bontenbal M, et al: Cell biological factors associated with the response of breast cancer to systemic treatment. *Cancer Treat Rev* 19:45-63, 1993.

24. Wright C, Nicholson S, Angus B, et al: Relationship between c-erbB-2 protein product expression and response to endocrine therapy in advanced breast cancer. *Br J Cancer* 65:118-121, 1992.

25. Carlomagno C, Perrone F, Gallo C, et al: c-erbB2 overexpression decreases the benefit of adjuvant tamoxifen in early-stage breast cancer without axillary lymph node metastases. *J Clin Oncol* 14:2702-2708, 1996.

26. Allred DC, Clark GM, Tandon AK, et al: HER-2/neu in node-negative breast cancer: prognostic significance of overexpression influenced by the presence of in situ carcinoma. *J Clin Oncol* 10:599-605, 1992.

27. Gusterson BA, Gelber RD, Goldhirsch A, et al: Prognostic importance of c-erbB-2 expression in breast cancer. *J Clin Oncol* 10:1049-1056, 1992.

28. Muss HB, Thor AD, Berry DA, et al: c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer. *N Engl J Med* 330:1260-1266, 1994.

29. Seidman AD, Baselga J, Yao T-J, et al: HER-2/neu over-expression and clinical taxane sensitivity: a multivariate analysis in patients with metastatic breast cancer (mbc) (abstract). *Proc Am Soc Clin Oncol* 15:#80, 1996.

30. Blagosklonny MV, Schulte TW, Nguyen P, et al: Taxol induction of p21WAF1 and p53 requires c-raf-1. *Cancer Res* 55:4623-4626, 1995.

31. Blagosklonny MV, Schulte T, Nguyen P, et al: Taxol-induced apoptosis and phosphorylation of bcl-2 protein involves c-Raf-1 and represents a novel c-Raf-1 signal transduction pathway. *Cancer Res* 56:1851-1854, 1996.

32. Ding A, Sanchez E, Nathan CF: Taxol shares the ability of bacterial lipopolysaccharide

to induce tyrosine phosphorylation of microtubule-associated protein kinase. *J Immunol* 151:5596-5602, 1993.

33. Waskiewicz AJ, Cooper JA: Mitogen and stress response pathways: MAP kinase cascades and phosphatase regualtion in mammals and yeast. *Curr Opin Cell Biol* 7:798-805, 1995.

34. Fendly BM, Winget M, Hudziak RM, et al: Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor to HER2/neu gene product. *Cancer Res* 50:1550-1558, 1990.

35. Drebin JA, Link VC, Weinberg RA, et al: Inhibition of tumor growth by a monoclonal antibody reactive with an oncogene-encoded tumor antigen. *Proc Natl Acad Sci USA* 83:9129-9133, 1986.

36. Pietras RJ, Fendly BM, Chazin VR, et al: Antibody to HER-2/neu receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* 9:1829-1838, 1994.

37. Baselga J, Norton L, Coplan K, et al: Antitumor activity of placitaxel in combination with anti-growth factor receptor monoclonal antibodies in breast cancer xenografts (abstract). *Proceedings American Association for Cancer Research* 35:2262, 1994.

38. Carter P, Presta L, Gorman CM, et al: Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc Natl Acad Sci USA* 89:4285-4289, 1992.

39. Baselga J, Tripathy D, Mendelsohn J, et al: Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. *J Clin Oncol* 14:737-744, 1996.

40. Hancock MC, Langton BC, Chan T, et al: A monoclonal antibody against the c-erbB-2 protein enhances the cytotoxicity of cis-diamminedichloroplatinum against human breast and ovarian tumor cell lines. *Cancer Res* 51:4575-4580, 1991.

41. Arteaga CL, Winnier AR, Poirier MC, et al: p185HER2 Signaling enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. *Cancer Res* 54 3758-3765, 1994.

42. Pegram M, Lipton A, Pietras R, et al: Phase II study of intravenous recombinant humanized anti-p185 HER-2 monoclonal antibody (rhuMAb HER-2) plus cisplatin in patients with HER-2/neu overexpressing metastatic breast cancer (abstract). *Proc Am Soc Clin Oncol* 14:124, 1995.

43. Levitzki A, Gazit A: Tyrosine kinase inhibition, an approach to drug development. *Science* 267:1782-1787, 1995.

44. Osherov N, Gazit A, Gilon C, et al: Selective inhibition of the epidermal growth factor and Her2/neu receptors by tyrphostins. *J Biol Chem* 268:11134-11142, 1993.

45. Fry DW, Kraker AJ, McMichael A, et al: A specific inhibitor of the epidermal growth factor receptor tyrosine kinase. *Science* 265:1093, 1994.

46. Bos M, Mendelsohn J, Kim YM, et al: A tyrosine kinase inhibitor prevents ligand-induced receptor activation and inhibits growth of cancer cell lines expressing the epidermal growth factor receptor (abstract). *Proc Am Assoc Cancer Res* 37:305, 1996.

47. Zhang L, Hung M-C: Sensitization of HER-2/neu-overexpressing non-small cell lung cancer cells to chemotherapeutic drugs by tyrosine kinase inhibitor emodin. *Oncogene* 12:571-576, 1996.